Effects of Dietary Nitrate Supplementation on Blood Pressure Reduction,

A Systematic Review and Meta-Analysis

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Abstract

Background

High blood pressure is the greatest risk factor for diseases such as hypertension, stroke, and cardiovascular disease. Blood pressure control has become a global public health priority. Nitric oxide (NO), which can be converted from nitrate, has been reported to reduce blood pressure. Clinical trials have been conducted to test the effect of dietary intake rich in nitrate on reduction of blood pressure and the results have been inconsistent. We aimed to conduct a meta-analysis of randomized clinical trials to evaluate the impact of dietary nitrate on blood pressure (BP) reduction.

Methods

Pubmed, EMBASE, and Web of Science were systematically searched from inception until July 2018. We included the randomized clinical trials that examined the effect of dietary nitrate supplementation like beetroot supplementation in reducing systolic blood pressure (SBP) and diastolic blood pressure (DBP) compared to taking placebo or low nitrate supplementation. A random-effects meta-analysis was conducted to evaluate the intervention of nitrate diet on the control of BP.

Results

A total of 41 trials including 733 participants (with 5 to 65 participants per study) were eligible. The duration of each intervention ranged from 3 hours to 12 weeks with the median value of 3 days (25%-75% quartile range equal to 1-7 days). 21 out of 41 studies showed that dietary nitrate was associated with a significant decline in SBP compared to placebo, while only 12 out of the 21 above studies indicated that dietary nitrate was also associated with a significant DBP reduction. There were 3 studies where dietary nitrate significantly reduced in DBP but not SBP. Overall, blood pressure (BP) reductions were -3.12 mm Hg (95% CI, -4.59 to -1.54) for systolic and -1.43 mm Hg (95% CI, -2.55 to -0.30) for diastolic. The greater effect size on BP reduction was found among the groups with the interventions of beetroot juice and nitrate-depleted placebo (MD in SBP for beetroot juice=-4.68 mmHg, 95% CI: -6.57 to -2.01, MD in DBP for beetroot juice=-2.35 mmHg, 95% CI: -3.86 to -0.84, MD in SBP for nitrate-depleted placebo=-4.40 mmHg, 95% CI: -6.79 to -2.01, and MD in DBP for nitrate-depleted placebo=-2.10 mmHg, 95%

CI: -3.85 to -0.35). The higher impact of beetroot and inorganic nitrate supplementation on BP reduction found in older subjects was mostly associated with the structural changes in their arteries and especially with large artery stiffness. Our meta-regression analysis also demonstrated a significant negative relationship between age and the treatment effect on DBP after beetroot and inorganic nitrate consumption (slope=-0.0696; p=0.031).

Conclusion

Overall, dietary nitrate showed significant effect in reducing SBP, while that in reducing DBP is less consistent. This study provided insight to potential complementary therapies for acute blood pressure lowering effect in populations, particularly when internal source of NO suffers from dysfunction. Dietary nitrate is an essential supplement for the prevention and treatment of hypertension. Public health leaders should implement effective interventions that incorporate individual behavior changes to increase intake of nitrate diet and environmental changes to encourage and promote greater access to healthier food.

Keywords

Beetroot, inorganic nitrate, blood pressure, hypertension, systematic review, meta-analysis, meta-regression

Introduction

Noncommunicable diseases (NCDs), also known as chronic disease, leads to the death of 41 million people each year, equivalent to 71% of all deaths globally according to the World Health Organization (WHO). A major challenge to meeting the 2030 Sustainable Development Goals (SDGs), NCDs have four key metabolic risk factors: 1) raised blood pressure (hypertension), 2) overweight, 3) hyperglycemia (high blood glucose levels) and 4) hyperlipidemia (high levels of fat in the blood) (1). By changing the structure of the arteries, hypertension is a major factor in stroke, cardiovascular disease, kidney failure, other diseases (2, 3). Hypertension as the leading risk factor contributed to about 40% of people worldwide according to WHO and 1/3 of U.S. adults based on CDC (4,5). In both developing and developed countries, 51% of stroke and 45% of ischaemic heart disease deaths are due to high systolic blood pressure (SBP) (4). About 50 million people in the United States and one billion people globally suffer hypertension (6). People who are normotensive at age 55 have a 90% lifetime risk for developing hypertension (7). For individuals older than 50 years, SBP greater than 140 mmHg is a more important cardiovascular disease (CVD) risk factor than DBP; for individuals between 40–70 years of age, each increment of 20 mmHg in SBP or 10 mmHg in DBP can double the risk of CVD across the BP range from 115/75 to 185/115 mmHg (7).

Therefore blood pressure (BP) control has become a worldwide public health priority, and has been emphasized on the public health agenda. May 17th is marked as World Hypertension Day (5). WHO target is to reduce the relative prevalence of raised blood pressure by 25% in the next 7 years (8). Although hypertension is not an inevitable disease, once it develops it often requires costly, lifelong treatment with medicines. Thiazide-type diuretics, called water pills acting on the kidneys to help the body eliminate sodium and water and reduce blood volume, either alone or combined with other types of drugs, are recommended to patients with uncomplicated hypertension (7, 9). Most patients with hypertension require two or more antihypertensive drugs to achieve their target BP (<130/80 mmHg or < 140/90 mmHg for patients with chronic kidney disease or diabetes respectively) (7). When BP becomes >20/10 mmHg above the target BP other antihypertensive drug classes such as angiotensin receptor blockers, calcium channel blockers, and angiotensin-converting enzyme inhibitors may be needed (7). But patients may still not be able to reach their goal BP with the combinations of the above medicines and alpha blockers,

alpha-beta blockers, beta blockers, aldosterone antagonists, renin inhibitors, vasodilators, and central-acting agents may be prescribed by their doctors for the treatment (9).

Antihypertensive drugs only adequately maintain BP in about 50% of hypertensive cases (10). Lifestyle changes play a very important role in the primary prevention of hypertension. Regular physical activity through at least 150 minutes a week of moderate aerobic activity or 75 minutes a week of vigorous aerobic activity, a combination of moderate and vigorous activity, interval training in which short bursts of intensive activity with short recovery periods of lighter activity alternatively taken, or at least two days a week of muscle-strengthening exercises, would help to lower the BP as well (9). It was demonstrated that patients receiving exercise interventions gained a modest reduction in both systolic (-3.1 mmHg, 95%CI: -0.7 to -5.5) and diastolic (-1.8 mmHg, 95% CI: -0.2 to -3.5) blood pressure compared to people without interventions (13). Increase physical activity not only aids in keeping the body weight under control, but also helps to manage stress and reduce the risk of other health problems. It is reported that each kilogram of weight lost may reduce BP by about 1 mmHg (9).

Meanwhile, other lifestyle changes and home remedies have been explored by clinicians as reducing salt/sodium in daily diet by putting down the saltshaker and eating less processed foods such as canned soups or frozen dishes may contribute to 2-8 mmHg reduction in SBP (7, 9). Limiting the intake of to one drink a day for women, and up to two drinks a day for men is recommended, since alcohol can raise the BP even in healthy condition. One drink means 12 ounces of beer, 5 ounces of wine or 1.5 ounces of 80-proof liquor (7,9). Moderation of alcohol consumption could lower SBP 2-4 mm Hg (7). Smoking is associated with poor cardiovascular and pulmonary outcomes by injuring blood vessel walls and accelerates the process of the buildup of plague in the arteries (9, 13). Regular physical activity, plenty of sleep, refusal of extra tasks, maintaining good relationships, releasing negative thoughts, and remaining patient and optimistic and practicing healthy coping techniques such as muscle relaxation, deep breathing and meditation helps manage stress (9). Relaxation interventions were associated with statistically significant reductions in systolic (-3.7 mmHg, 95%CI: -1.3 to -6.0) and diastolic (-3.5 mmHg, 95%CI: -1.9 to -5.1) BP (13). Monitoring BP at home helps to keep a closer eye on BP on a daily basis to see if medication working well and to alert the patients and doctors to potential complications (9). Some supplements including fiber, such as blond psyllium and wheat

bran, folic acid, minerals such as magnesium, calcium, and potassium, Omega-3 fatty acids, the products widen blood vessels (vasodilators) such as cocoa, coenzyme Q 10, L-arginine or garlic, vitamin D might help lower BP (9). For women, menopausal hormone therapy not raising BP should be considered as a form of contraception instead of oral contraceptives that increase the risk of hypertension with duration of use. Pregnant women with hypertension should be followed very carefully due to the increased risks to both mother and fetus. Methyldopa, beta-blockers, and vasodilators are preferred medications for the safety of the fetus (7). Intake of caffeine has long being associated with raised BP and demonstrated a dose-related increase of 5-15 mmHg systolic and 5-10 mmHg diastolic BP for a few hours. Though caffeine varies based on the different beverages, typically coffee contains 60-120 mg caffeine per 150 ml cup compared to tea (20 – 40 mg per 150 ml cup) and cola drinks (30-50 mg per 330 ml can). Caffeine acts like an adenosine receptor antagonist that leads to vasoconstriction and raised BP. Its half-life in the body is about five hours (13).

Dietary Approaches to Stop Hypertension (DASH) and being physically active are also recognized as effective strategies for lowering the BP and maintaining a healthy weight (11). DASH highlights the importance of eating healthier foods such as fruits, vegetable, fish, less saturated fat and trans-fat dairy foods, whole grains, poultry, fish, and more potassium to help prevent and control high BP (9,12). Adopting a DASH eating plan may result in 8-14 mm Hg reduction in SBP (7). Even though DASH emphasizes the beneficial effects of increased consumption of nitrate/nitrite-rich food products such as fruits, green leafy and root vegetables on BP reduction, and clinical trials have also been conducted to test the effect of dietary intake rich in nitrate on the reduction of BP, overall the results are inconsistent (14). The objective of this paper is to conduct a systematic review and meta-analysis of randomized clinical trials to evaluate the impact of dietary nitrate on BP reduction in humans. The results will inform whether inorganic nitrate including dietary nitrate mainly in beetroot supplement can be considered to be an effective intervention for the prevention of hypertension.

Background

It is documented that the dietary nitrate is first absorbed in the upper gastrointestinal tract and then about 25% of the absorbed nitrate is delivered to the salivary gland and secreted into saliva where is reduced to nitrite by commensal anaerobic bacteria on the tongue (15). Acid stomach

protonated part of the nitrite into nitrous acid ($NO_2^- + H^+ \rightarrow HNO_2$), and then decomposed to a variety of nitrogen oxides such as NO, nitrogen dioxides (NO_2), and dinitrogen trioxide (N_2O_3) ($2\ HNO_2 \rightarrow N_2O_3 + H_2O$, $N_2O_3 \rightarrow NO + NO_2$) (15). The leftover nitrate/nitrite is efficiently absorbed in the intestine and enters the bloodstream, where mixing with nitrate from NO synthases (NOS), the main endogenous sources (16). Part of the nitrate is excreted in the kidney, rest of the nitrate is actively taken up by the salivary glands and circulated in the nitrate-nitrite-NO pathway (16).

The NO as an endogenous effector molecule circulating in the body is originated from three resources: the diet, endogenous NOS enzyme, and the drugs (Figure 1) (15). NO donor drugs can directly produce NO to reduce BP (15). Dietary nitrate/nitrite and NOS producing NO are linked to the cyclic guanosine monophosphate (cGMP) functions of NO by mediating local vasodilatation (15,17). cGMP as the second messenger of NO is crucial in blood pressure regulation and in the etiology of hypertension (17).

Methods

Literature search strategies

We systematically searched PubMed, Web of Science, Embase and PsycInfo for randomized clinical trials (RCTs) that evaluated the effect of dietary nitrate supplementation in reducing systolic blood pressure (SBP) and diastolic blood pressure (DBP) compared to taking placebo or low nitrate supplementation from inception to July 2018. The search was conducted based on predefined search terms (dietary, inorganic, nitrate, beetroot, blood pressure, and hypertension). Additionally, we manually searched for studies listed in references, in case there were potential studies not captured by the database search strategy. We also hand-searched the studies published most recently. The search was limited to articles written in English. The present systematic review was conducted according to the established PRISMA guidelines (18).

Inclusion and exclusion criteria

The inclusion and exclusion criteria for all included papers were established by two researchers. The systematic review and meta-analysis only included primary research published in peer-reviewed journals that: (1) were randomized, parallel or crossover, placebo-controlled designs that delivered treatment of nitrate or beetroot supplementation, (2) included adult male and

female participants (age greater than18 year-old) with or without health comorbidities, (3) investigated the treatment effects on changes in both SBP and DBP. Studies were excluded from the meta-analysis if the outcomes were incomplete, for instance, only reported changes in average outcomes between treatment and control groups. We further excluded the studies that delivered the above supplementation alongside another intervention (e.g. exercise) if the interventions were different between groups.

Study selection and data extraction

Study selection was conducted in 3 steps. First, the titles and abstracts of studies identified in our literature search were independently reviewed by 2 reviewers. Second, the full texts of studies retrieved in the initial screening were independently reviewed by 2 reviewers and disagreements were reconciled. Third, characteristics of studies that met inclusion criteria and exclusion criteria were extracted independently by 3 reviewers, including author, year of publication, study design, study's country, sample size, designs of treatment (duration, dosage and frequency), mean and standard deviation of the outcomes in changes adjusted for baseline measurements. Demographic information of participants from included studies would be extracted by 3 reviewers, including age, sex, and health comorbidities.

If the outcomes of interest were measured at multiple time points for one cohort, all related outcomes were extracted for potential subgroup analysis. If more than one type of dietary nitrate intervention were examined within the same trial, for example, high nitrate diet, and the combination of high nitrate diet and beetroot juice, all types of interventions were extracted and analyzed separately.

Quality assessment

The quality of each study was assessed using the Cochrane Risk of Bias Tool to assess the following domains: selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel to which intervention a participant received), detection bias (blinding of outcome assessment), attrition bias (completeness of outcome data, including attrition and exclusions from the analysis), and reporting bias (selective outcome reporting) (19).

Statistical analyses

Meta-analysis using random-effects model was conducted to evaluate the impact of dietary nitrate intervention on the changes of blood pressure (in SBP and DBP, respectively) between treatment and control group. Data were presented as mean difference (MD) of changes in SBP and DBP (in mm Hg) with corresponding 95% confidence interval (CI). The pooled MD is considered statistically significant if 95% CI did not contain 0. Individual and pooled estimates were illustrated using forest plots for both SBP and DBP. I^2 statistic was calculated to quantify the proportion of between-study heterogeneity attributable to variability in the association rather than sampling variation, and significant heterogeneity was considered existing if p values corresponding to I^2 statistic is smaller than 0.05. Potential publication bias was evaluated using Egger's regression test. Subgroup analysis were performed to assess whether changes in diastolic and systolic BP were influenced by study duration (<3 vs. ≥3 days), the types of intervention (beetroot supplementation vs. other inorganic nitrate), choice of placebo (nitrate-depleted beetroot vs. others), and specific population such as age and BMI. Random-effects meta-regression was implemented to evaluate the impact of age, dosage, and sample size on changes in BP outcomes. Sensitivity analysis were conducted to evaluate the robustness of the results. All meta-analyses were conducted using 'meta', 'metafor', and 'mvmeta' in R (3.4.4 version).

Measurement of treatment effects

Net treatment effects on SBP and DBP respectively were determined by subtracting the mean changes of each outcome from baseline to the last available measurement in the control group from the corresponding mean changes in the active treatment group (20). We will refer to the treatment effects as mean difference in changes in SBP or DBP from here on. Standard errors (SEs) of the treatment effects were obtained either directly from the value available in the study, or calculated from SDs or CIs when the sample size were described in each trial.

Results

Search results

Initial database search yielded a total of 4,511 records as shown in the flow chart (Figure 2). After excluding letters or reviews and eliminating duplicates, 3,322 remained. After the abstract screening, 3,218 studies were not relevant and therefore excluded. The 104 remaining reports were retrieved in full-text. A total of 41 trials including 733 participants (with 5 to 65

participants per study) were enrolled. The main characteristics of the studies included are shown in Table 1. The duration of each intervention ranged from 3 hours to 12 weeks with the median value of 3 days (25%-75% quartile range equal to 1-7 days). 21 out of 41 studies showed that dietary nitrate was associated with a significantly decline in SBP compared to placebo, while only 12 out of the 21 above studies indicated that dietary nitrate was also associated with a significantly DBP reduction. There were 3 studies where dietary nitrate significantly lower DBP but not SBP. Finally, a total of 14 articles were included in our meta-analysis, including 4 for randomized parallel and 10 for randomized crossover studies.

Study characteristics

The study characteristics of the studies included in the systematic review and meta-analysis were shown in Table 1.

Table 1: Main study characteristics of the studies included in the systematic review and meta-analysis.

Reference	Year	Country	No. of participants	Age	Male (%)	BMI (kg/m^2)	Healthy Condition	Study Duration	Wash out Period
Miller et al(21)	2012	USA	8	72.5	37.5	28.5	healthy	3 days	3-12 days
Hobb et al(22)	2013	UK	23	31	100	23.3	healthy	6 hours	
Kim et al(23)	2015	USA	12	22	100	25	healthy	1 day	5 days
Bondonno et al(24)	2015	Australia	19		36.8		healthy	7 days	14 days
Bailey et al(25)	2010	UK	7	28	100	25	healthy	6 days	10 days
Velmurugan et al(26)	2017	UK	65	53.3	36	26.8	hypercholesterolemia	6 weeks	
Bourdillion et al(27)	2015	Switzerland	34 12	31	100		healthy	3h 3 days	5 days
Lansley et al(28)	2010	UK	9	22	100	22.1	healthy	6 days	10 days
Kelly et al(29)	2012	UK	12	64	50	23.2	healthy	2.5 days	72 hours

Vanhatalo et al(30)	2010	UK	8	29	62.5	23.4	healthy	15 days	10 days
Jovanovski et al(31)	2015	Canada	24	24.5	40.7	22.8	healthy	7 days	7 days
Jonvik et al(32)	2016	Netherland	18	28	61.1	23	healthy	300 min	7 days
Larsen et al(33)	2006	Sweden	17	27.4	88.2	24	healthy	3 days	10 days
Silva et al(34)	2016	Brazil	5	58	80	22.8	healthy	3 hours	
Houston et al(35)	2014	USA	30		63.6		hypertension	4 hours	3 weeks
Haider et al (36)	2014	UK	18	21	100	22	healthy	7days	
Coles et al(37)	2012	Australia	30	42.5	50	28.2	healthy	24hours	10 days
Bloomer et al(38)	2010	US	10	26	100	27.5	healthy	1 week	
Webb et al(39)	2008	UK	14	25.5	64.3	22.5	healthy	24hours	
Kapil et al(40)	2015	UK	64	56.95	40.6	26.65	hypertension	24 hours 24 hours 4 weeks	2 weeks
Ashworth et al(41)	2015	UK	19	20	0	22.5	healthy	1 week	3 weeks
Lansley et al (42)	2011	UK	9	21	100	24.3	healthy	3 hours	48-72 hours
Bahra et al(43)	2012	UK	14	27.9		24.5	healthy	3 hours	
Bond et al(44)	2014	USA	13	20.9	0	23.5	healthy	3-7 weeks	
Leong et al(45)	2015	Australia	23	67	26.3	29.1	COPD	3 days	
Wong et al(46)	2014	Australia	37	58.5	54.1	27.2	overweight	12 weeks	24 hours
Bond Jr. et al(47)	2013	USA	12	20.7	0	23.6	healthy	1-2 week	
Bailey et al(48)	2015	UK	7	21	100	26	healthy	9 days	10 days
Eggbeen et al(49)	2016	USA	19	69	15	32.9	heart failure	1 week	3-7 days

							with preserved ejection fraction		
				69	15	32.9		1 day	
Lee et al(50)	2015	USA	14	22	100	23.4	healthy	15 days	2 weeks
Levitt et al(51)	2015	USA	7	22	100	25	healthy	3days	
Kapil et al(52)	2010	UK	20	22.5	40	22.5	healthy	24 hours	≤24 hours
			6	28.8	40	24.5	healthy	24 hours	≤24 hours
			9	25.1	40	26.5	healthy	24 hours	≤24 hours
Berry et al(53)	2015	USA	15	69.6	80	29.2	COPD	48hours	
Bailey et al(54)	2009	UK	8	26	100	25.3	healthy	6 days	10 days
Larsen et al(55)	2009	Sweden	9	30	88.8		healthy	2 days	7 days
Hobbs et al(56)	2012	UK	18	31.4	100	24.4	healthy	24 hour	7 days
			14	25.1		23.5	healthy		
Gilchrist et al(57)	2013	UK	27	67.2	66.7	30.8	type 2 diabetes	2 weeks	4 weeks
Cermak et al(58)	2012	Netherlands/Canada	12	31	100	23.3	healthy	6 days	14 days
Raubenheimer et al(59)	2017	Australia	12	41.7	25.7	72	healthy	3 days	2 weeks
Kerley et al(60)	2018	UK	20	65	30.7	168	uncontrolled hypertension, diverse anti-hypertensive regimens	7 days	24 hours
Eglin et al(61)	2017	UK	13	34.5	30.8	27.1		3 days	7 days

Types of treatments / placebos

The included studies investigated the effects of dietary nitrate including beetroot supplementation (juice/extract), green vegetables (e.g. spinach), combined extracts of green vegetables and beetroot supplementation, inorganic nitrate compounds (e.g. Sodium nitrate), and NO dietary supplement marketed as a new ‘‘real nitric oxide” molecule (2-(nitrooxy)ethyl 2-amino-3-methylbutanoate). The placebo involved in lower nitrate (e.g. asparagus soup), the negligible nitrate content (e.g. orange juice/black-currant juice), and the nitrate-depleted BR juice, though some studies did not describe the details of placebo.

Demographic characteristics for meta-analysis

The demographic characteristics of the 14 trials are shown in Table 1 (15, 19, 20, 23, 25, 31, 32, 34, 35, 40, 45, 51, 54, and 55). The majority of these trials recruited nonsmoking, healthy, young participants with the median age of 48 years old ranged from 27 to 62 years old. Half of the participants were men. The mean BMI of the participants was 27 kg/m^2 with range from 25 to 28 kg/m^2. Two studies was conducted in hypertension individuals, one in overweight individuals, one in hypercholesterolemic individuals, and one in diabetes participants. In total, the 14 cohorts included 343 participants with 7 to 65 participants per study were enrolled in final meta-analysis. The duration of each intervention ranged from 24 hours to 12 weeks with the median value of 7 days (25%-75% quartile range equal to 3-13 days).

Study quality

The RCTs were evaluated by two reviewers (SS, QH) using the Cochrane risk of bias assessment tool (19), which evaluated 6 domains including random assignment, allocation concealment, blinding of participants, incomplete outcome data, selective outcome reporting, and other sources of bias. The assessment of “yes,” “no,” or “unclear” was assigned to each domain for respective designation of a low, high, or unclear risk of bias. If “unclear” or “no” was assigned to one or less domains, the study was evaluated as having a low risk of bias. If over four domains were assigned “unclear” or “no”, the study was evaluated as having a moderate risk (Figure 3).

Adverse events

It was reported that adverse events occurred in 16 studies. One study reported gastrointestinal and headache, and one trial had mild bloating complaints. The most common side effect

documented in 12 studies was beeturia (red urine) and red stools due to the beetroot supplementation consumption.

Meta-analyses

Overall, beetroot supplementation was associated with a significant reduction in SBP and DBP. In specific, the reduction in SBP was significantly higher in beetroot and inorganic nitrate supplementation than in the control groups (MD=-3.07, 95% CI: -4.59 to -1.54) without heterogeneity (I^2=23%, p=0.17) (Figure 4), and the reduction in DBP was significantly higher in beetroot and inorganic nitrate supplementation than in the control groups (MD=-1.43, 95% CI: -2.55 to -0.30) without heterogeneity (I^2=21%, p=0.2) (Figure 5).

Subgroup Analyses

Table 2 presented the results from subgroup analysis using random-effects meta-analysis (Supplemental figures 1-6).

Table 2: Meta-analysis of random clinical trials reporting the effects of beetroot juice and inorganic nitrate supplementation on blood pressure.

	Studies	**Effect size (mean difference (95% CI))**	**P value***	**I^2**
	n	mmHg		
Study duration				
SBP				
≤ 3 days	10	-3.26 (-5.67, -0.85)	0.27	18
> 3 days	9	-2.91 (-5.00, -0.83)	0.14	35
DBP				
≤ 3 days	10	-1.17 (-3.08, 0.74)	0.13	35
> 3 days	9	-1.61 (-3.01, -0.21)	0.36	9
Age				
SBP				
<50 years old	8	-2.18 (-4.12, -0.24)	0.86	0
≥ 50 years old	11	-3.52 (-6.05, -1)	0.05	45
DBP				
<50 years old	8	0.11 (-1.56, 1.79)	0.63	0
≥ 50 years old	11	-2.15 (-3.51, -0.79)	0.25	20
Type of Treatment				
SBP				

Beetroot juice	11	-4.68 (-6.57, -2.79)	0.32	13
Inorganic nitrate	8	-1.12 (-3.02, 0.77)	0.85	0
DBP				
Beetroot juice	11	-2.35 (-3.86, -0.84)	0.2	25
Inorganic nitrate	8	0.06 (-1.47, 1.60)	0.86	0
Type of Placebo				
SBP				
Nitrate-depleted beetroot	8	-4.40 (-6.79, -2.01)	0.14	36
Others	11	-1.57 (-3.33, 0.19)	0.85	0
DBP				
Nitrate-depleted beetroot	8	-2.10 (-3.85, -0.35)	0.1	42
Others	11	-0.35 (-1.82, 1.11)	0.74	0
Healthy Conditions				
SBP				
Healthy	12	-2.27 (-4.12, -0.43)	0.94	0
Disease	7	-3.60 (-6.62, -0.59)	0.01	64
DBP				
Healthy	12	-0.40 (-1.92, 1.12)	0.7	0
Disease	7	-2.02 (-3.77, -0.28)	0.07	48
BMI				
SBP				
<25 kg/m^2	4	-2.52 (-4.98, -0.07)	0.72	0
>=25 kg/m^2	15	-3.06(-5.06, -1.05)	0.08	36
DBP				
<25 kg/m^2	4	0.23 (-1.81, 2.27)	0.35	9
>=25 kg/m^2	15	-1.98 (-3.15, -0.80)	0.36	8

*: P value corresponding to heterogeneity test

When the meta-analysis stratified by type of intervention, the intervention of beetroot juice resulted in significant reduction in both SBP and DBP (MD=-4.68 mmHg, 95% CI: -6.57 to -2.01, and MD=-2.35 mmHg, 95% CI: -3.86 to -0.84, respectively), while the reduction was not significant in inorganic nitrate. Although the reduction for longer study duration (> 3 days) in DBP was significantly higher in the treatment groups than in the control groups (MD=-1.61 mmHg, 95% CI: -3.01 to -0.21), the reduction in DBP for shorter study duration (≤ 3 days) was not significantly different between the two groups. The reduction in DBP stratified by age indicated that the younger participants (<50 years old) was not significantly different, however the reduction for older participants (≥ 50 years old) in DBP was significantly higher in the treatment groups than in the control groups (MD=-2.15 mmHg, 95% CI: -3.51 to -0.79). In

addition, the reduction in DBP for greater BMI (>=25 kg/m^2) was significantly higher in the treatment group than in the control group (MD=-1.98 mmHg, 95% CI: -3.15 to -0.80), but there was no significant difference in smaller BMI (<25 kg/m^2) between the two groups. The subgroup analysis by healthy conditions indicated a significant reduction in DBP for disease participants (MD=-2.02 mmHg, 95% CI:-3.77 to -0.28), but no significant reduction for healthy participants.

A significant reduction in both SBP and DBP (MD=-4.40 mmHg, 95% CI: -6.79 to -2.01, and MD=-2.10 mmHg, 95% CI: -3.85 to -0.35) was also observed during the stratification by type of placebo in the trials using nitrate-depleted beetroot, while the reduction was not significant in other controls. The stronger effect size observed in the trials that used nitrate-depleted beetroot as a placebo compared with other interventions demonstrated the potential nitrate-dependent effects on the blood pressure.

Publication bias and heterogeneity

An overall symmetric distribution of the studies around the mean effect size was observed in funnel plots for both SBP and DBP, indicating a low risk for publication bias (Supplemental Figures 7-8). Egger's regression test confirmed that nonsignificant publication bias for both SBP and DBP (P = 0.19 and 0.72 respectively). There was moderate heterogeneity for SBP (I^2 = 23%, p = 0.17) and for DBP (I^2 = 21%, p =0.20) in the meta-analysis models.

The subgroup analysis (Table 2) indicated that trials with a longer duration (> 3 days days), older participants (≥ 50 years old), overweight participants, and those that used nitrate-depleted beetroot as placebos might be potential sources of heterogeneity for SBP outcomes. A shorter duration (≤ 3 days), older participants (≥ 50 years old), and those that used nitrate-depleted beetroot as placebos might be potential sources of heterogeneity for DBP outcomes.

Meta-regression

A meta-regression analysis showed that mean changes in SBP were directly affected by its daily dosage of nitrate (P < 0.05); changes in DBP were not affected by the daily dosage of nitrate but were negatively correlated with the age of participants (Table 3).

Table 3: Meta-regression of potential moderators of blood pressure changes in response to beetroot supplementation.

	Slope	95% CI	P value	Q
SBP				
Age, years	-0.026	-0.121, 0.0694	0.59	0.285
Daily dosage of nitrate, mM	0.386	0.0224, 0.75	0.038	4.328
DBP				
Age, years	-0.0696	-0.133, -0.0066	0.031	4.68
Daily dosage of nitrate, mM	0.29	-0.0259, 0.606	0.072	3.24

Sensitivity analyses

Sensitivity analyses were conducted to evaluate whether changes in diastolic and systolic BP were influenced by potential disease cohorts. A random-effects model was applied to each subgroup to obtain the pooled estimate of the mean difference (Table 4). The overall effect of intervention on changes in SBP yielded similar results when excluding disease cohorts. However, the results became non-significant when excluding the hypertension cohorts, which indicated hypertension population would result in the potential differences between groups in terms of changes in DBP.

Table: Sensitivity analysis to evaluate the effects of potential modifying factors on the pooled systolic BP effect size.

Model (References)	Number of studies	Mean Difference SBP (95% CI) mmHg	I2 (SBP)	P Value* (SBP)	Mean Difference DBP (95% CI) mmHg	I2 (DBP)	P Value* (DBP)
Exclusion of 5 cohorts with disease participants	9	-2.27 (-4.12, -0.43)	0	0.94	-0.40 (-1.92, 1.12)	0	0.7
Exclusion of 2 cohorts with hypertension participants	12	-1.60 (-3.11, -0.09)	0	0.92	-0.32 (-1.45, 0.81)	0	0.88
Exclusion of 1 cohorts with hypercholesterolemia participants	13	-3.11 (-4.81, -1.40)	30	0.12	-1.81 (-3.04, -0.59)	17	0.25
Exclusion of 1 cohort with diabetes participants	13	-3.21 (-4.78, -1.65)	24	0.17	-1.44 (-2.64, -0.23)	25	0.16

Exclusion of 1 cohort with overweight participants	13	-3.71 (-5.08, -2.34)	4	0.41	-1.47 (-2.70, -0.25)	25	0.16

*: P value comes from heterogeneity I2

Sensitivity analyses were also conducted to evaluate whether changes in diastolic and systolic BP were influenced by the cohorts with gender. A random model was applied to each subgroup to obtain the pooled estimate of the mean difference (Table 5). Excluding trials with male only or female only did not change the results for either SBP (MD: -2.93 mmHg, 95% CI: -4.69 to -1.17 and MD: -3.00 mmHg, 95% CI: -4.61 to -1.40, relatively) or DBP (MD: -1.28 mmHg, 95% CI: -2.50 to -0.06 and MD: -1.45 mmHg, 95% CI: -2.63 to -0.26, relatively).

Table 5: Sensitivity analysis to evaluate the effects of potential modifying factors on the pooled systolic BP effect size.

Model (References)	**Number of studies**	**MD (95% CI) in SBP, *mmHg***	**I^2 (SBP)**	**P Value* (SBP)**	**MD (95% CI) in DBP, *mmHg***	**I^2 (DBP)**	**P Value* (DBP)**
Exclusion of 3 cohorts with male only	11	-2.93 (-4.69, -1.17)	0.09	34	-1.28 (-2.50, -0.06)	0.13	29
Exclusion of 1 cohort with female only	13	-3.00 (-4.61, -1.40)	0.13	28	-1.45 (-2.63, -0.26)	0.16	25

*: P value corresponding to heterogeneity test

Discussion

Our systematic review of 41 randomized studies indicated that the inorganic nitrate diet and supplement, separately or together, would lower resting BP compared to the control diet or no supplement interventions. The meta-analysis of 14 RCTs showed that beetroot and inorganic nitrate supplementation might be associated with significantly lower levels of both SBP and DBP (MD: -3.07 mmHg, 95% CI: -4.59 to -1.54 and MD: -1.43 mmHg, 95% CI: -2.55 to -0.30, respectively) compared to placebos.

According to subgroup analyses, the best effect of beetroot and inorganic nitrate supplementation on BP was observed when the duration of trial was > 3 days, while both SBP and DBP had significant reduction. Previous meta-analyses had reported the same term BP-lowering effects of

beetroot and inorganic nitrate, but Siervo and colleagues did not report the significant difference in DBP (10).

Our results also presented that the BP-lowering effects of beetroot and inorganic nitrate supplementation may be influenced by chronic disease. A significant reduction was observed in DBP after the interventions in diseased and overweight (BMI>=25 kg/m^2) participants than in healthy participants (MD=-2.02 mmHg, 95% CI:-3.77 to -0.28 and MD=-1.98 mmHg, 95% CI: -3.15 to -0.80, respectively). Additionally, hypertension participants were discovered to have a response to the intervention.

Our remarkable findings of subgroup analyses were interventions of beetroot juice and nitrate-depleted placebo contributed to greater effect size on BP reductions in both SBP and DBP (MD in SBP for beetroot juice=-4.68 mmHg, 95% CI: -6.57 to -2.01, MD in DBP for beetroot juice=-2.35 mmHg, 95% CI: -3.86 to -0.84, MD in SBP for nitrate-depleted placebo=-4.40 mmHg, 95% CI: -6.79 to -2.01, and MD in DBP for nitrate-depleted placebo=-2.10 mmHg, 95% CI: -3.85 to -0.35). In contrast, Zahra and colleagues only reported a weak effect size with nitrate-depleted placebo with moderate heterogeneity (62). Our studies demonstrated a potential nitrate-dependent effect on lowering the blood pressure.

A greater impact of beetroot and inorganic nitrate supplementation on BP reduction found in older subjects was mostly associated with the structural changes in their arteries and especially with large artery stiffness. It was reported the elderly had the high risk of increased pulse pressure due to the decreased DBP and increased SBP (63). Therefore, we might expect larger scope for the treatment to be effective for this outcome and type of subject, because their BPs are more extreme to begin with. Our meta-regression analysis also demonstrated that a significant negative relationship between age and the treatment effect on DBP after beetroot and inorganic nitrate consumption (slope=-0.0696; p=0.031). Though this finding was different from the previous reports by Ashor, Siervo and Zahra and their colleagues who had indicated that beetroot and inorganic nitrate had a lower effect on the older subjects due to the different level capacity to convert NO_3 into NO_2 (62,64,65). Beetroot and inorganic nitrate supplementation exerts its magnificent efforts to lower the BP among various participants.

Our meta-regression also identified daily dosage of nitrate as a potential moderator of SBP changes in response to beetroot and inorganic nitrate supplementation. We found a significant positive relationship between daily dosage of nitrate and the treatment effect on SBP after

intervention (slope=0.386; p=0.038). It was consistent with the recent reports by Siervo and Zahra and their colleagues (10, 62).

There are some limitations in the meta-analyses. Our broad inclusion criteria for selection and qualification of studies may have increased the heterogeneity between studies and made it difficult to apply our findings for specific patients, for example, those who have obesity. In addition, our searches were limited to English-language and published records, possibly increasing the risk of publication bias.

Conclusion

Our results showed a beneficial reduction in BP after relatively short-term beetroot supplementation, which contributes important new evidence that indicates the hypotensive properties of beetroot to be a potentially safe and cost effective nutritional therapy for managing hypertension, particularly when internal source of NO suffers from dysfunction. It may also potentially have important implications for the prevention of cardiovascular diseases. Meanwhile, it implies that dietary nitrate is an essential supplement for the prevention and treatment of hypertension that would be able to further prevent undesirable cardiovascular disease as well.

Implications for Public Health Leaders

While this meta-analysis had demonstrated the effectiveness of dietary nitrate in preventing and treating hypertension, there are many cultural and socioeconomic factors having an impact on whether people adopt and follow such a diet to control BP. According to the CDC, approximately 75% of Americans do not regularly consume enough fruits and more than 50% do not eat enough vegetables, while 64% eat too much saturated fat according to CDC (66). This behavior comes not only from the knowledge gaps in nutrition but also because of social determinants such as economic burden and limited access to healthier foods. The dietary problems are associated with health disparities and differentially influence people of different culture, race, age and educational level (66). Though the CDC has collaborated with the National Cancer Institute (NCI), the American Cancer Society (ACS) and three Department of Agriculture agencies to expand federal support for the national “5 A Day for Better Health” Program to help health organizations promote fruit and vegetable consumption, the importance of increase dietary nitrate has not been included.

Interventions to increased dietary nitrate should be one important step to prevent and control hypertension. Public health leaders (PHLs) play an important role in integrating this intervention into multifaceted programs (e.g. community-based programs, school-based programs and healthcare programs). Such programs should fall into 3 categories: 1) health promotion: to help individuals establish an active lifestyle and healthy eating behaviors by increasing nitrate consumption early in life and to maintain these behaviors throughout their lives; 2) primary prevention: to help individuals who have hypertension or NCDs, prevent or postpone the onset of disease by establishing more active lifestyles and healthier eating behaviors such as additional intake of nitrate; and 3) secondary prevention: to help individuals who already have hypertension or NCDs, control their conditions and prevent further disabilities by increasing their physical activity and developing much healthier eating habits (increasing dietary nitrate) (66).

In order to be most effective in the long run, healthy eating behaviors by consuming enough dietary nitrate combined with appropriate physical activity should be initiated in childhood and maintained throughout their life. Prevention interventions targeting older children and schools are equally important as interventions for adults who are inactive or have poor dietary behaviors even though they have not developed hypertension or NCDs.

All interventions should be appropriately applied to the target populations, and multiple strategies might be needed to reach different segments of the population. Interventions may address individuals, institutions, communities, policies, or the environment and should be implemented in various settings, such as schools, work settings, healthcare facilities and places of worship. Interventions would have the best results if they address not only the intentions of individuals, but also their social and physical environments, including the social networks and organizations that affect them.

For example, community-based programs implemented at a local level should work with local organizations to identify target populations and encourage entire community participation in a comprehensive approach that addresses the physical, social, cultural and political environments affecting the whole community. PHLs should consider the following steps for the interventions: 1) conduct community assessments to determine the dietary habits of residents, identify interventions that might help improve the incorporation of nitrate diets and identify community resources and potential designs that could help build up the interventions; 2) coordinate among

different stakeholders to improve dietary nitrate intake; 3) encourage the residents to participate in program planning, design, implementation and evaluation to encourage uptake; 4) educate public and policy makers about the importance of dietary nitrate; 5) promote broader social and environmental changes that complement individual change efforts such as promoting dietary nitrate food choices in restaurants, fast-food outlets, cafeterias, vending machines, recreation centers and 6) mass-media campaigns, cooperation with the food industry or local agriculture department, and implementation of legislation.

Healthcare programs should collaborate with community partners to establish an integrated approach to improve the intake of dietary nitrate. All children and adults enrolled in health care plans should have access to appropriate primary and secondary prevention care services related to healthy eating behaviors and physical activity. Promotion of healthy eating behaviors and physical activity could be part of the benefit packages in these plans. The plans could also include healthy eating behaviors especially dietary nitrate and physical activity as indicators in the surveillance data they collect. These indicators can be used to evaluate the effectiveness of interventions to increase the nitrate diet among patients in the healthcare system and their community partners.

In addition to convincing people to increase dietary nitrate and be more physically active, PHLs should work on creating environments, systems, and policies that increase public awareness about dietary nitrate and physical activity, eliminate barriers to the consumption of nitrate-rich foods, provide explicit support, reinforcement, and inducements to making healthy choices such as eating fruits or vegetables instead less healthy foods, change cultural and organizational norms, and establish themselves as partners in planning and decision-making on environmental and policy issues that affect people's eating and physical activity behaviors.

Finally, a well-built community coalition may also help create environments that serve as passive inducements to consuming a high nitrate diet for persons with hypertension by eating five servings of fruit and vegetables a day. There are a few potential advantages of working in a coalition for this purpose. A well-organized coalition has more power than an individual or organization alone to increase access to resources, create strategic alliances and support networks and to be a louder voice and to enhance the legitimacy of a cause. (67). For example, through the coalition, the parents with children can create new social networks that would strengthen their

ability to advocate for increasing the dietary nitrate; local departments can develop long-lasting bonds with professional organizations, media group and policymakers to create synergies; the local administrators can benefit from the collaborative bonds across racial and socioeconomic lines (67). Meanwhile, since developing healthy eating habits is a long complex process, the coalition can enhance coordination of services through multifaceted system approaches.

Overall, PHLs can implement effective interventions that incorporate the individual behavior changes to sustainable intake of nitrate diet and environmental changes to encourage and promote greater access to healthier food.

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Figures:

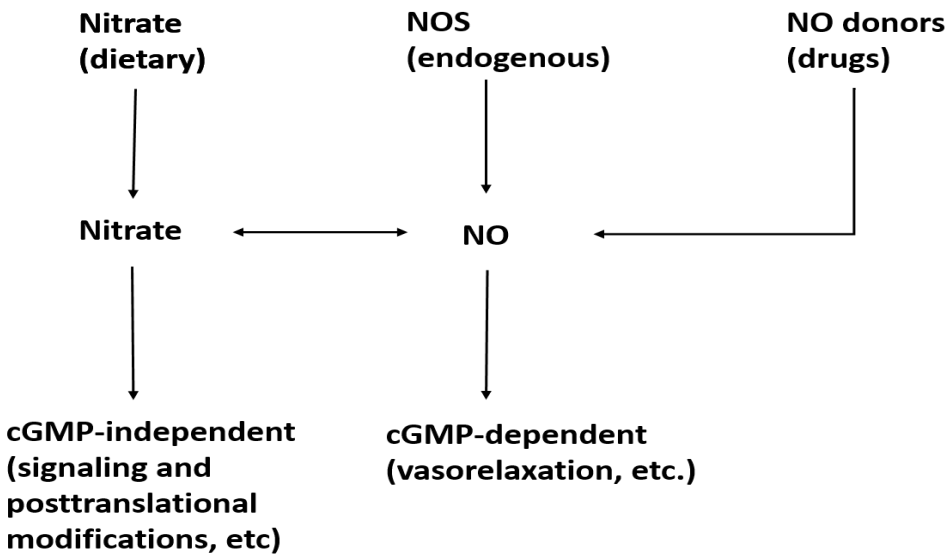


Figure 1: The NO pathways. NO is originated from the diet, endogenous NOS enzyme, and the drugs. Drugs can directly release NO, while NOS and dietary nitrate generate NO linked to the cGMP functions of NO.

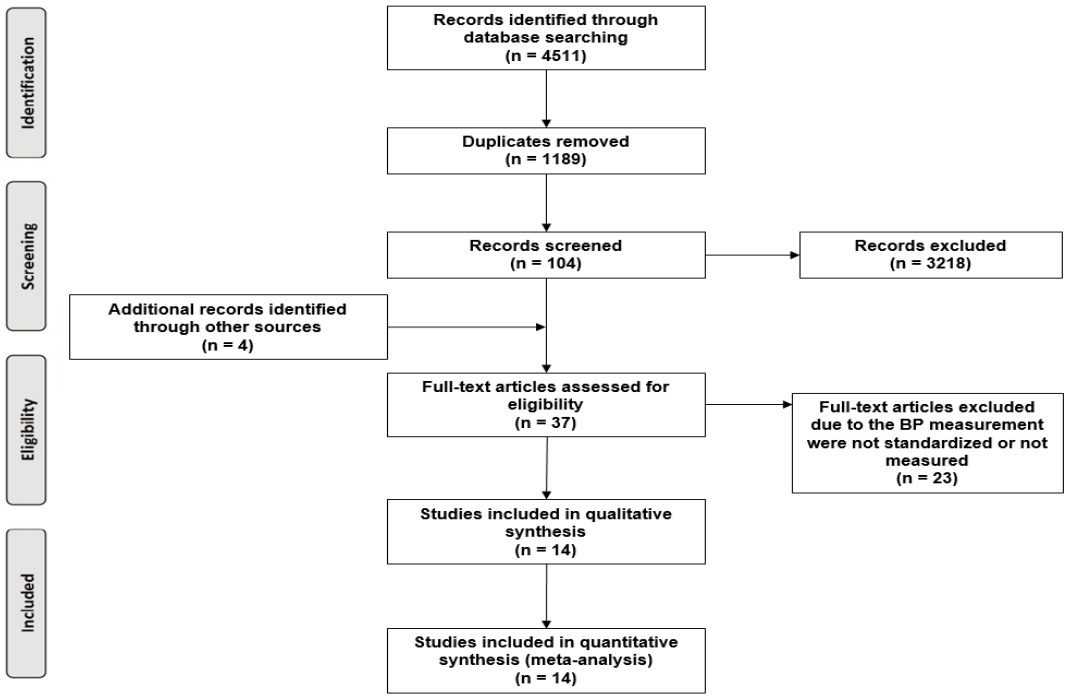


Figure 2: Flow chart of literature search. BP represents blood pressure.

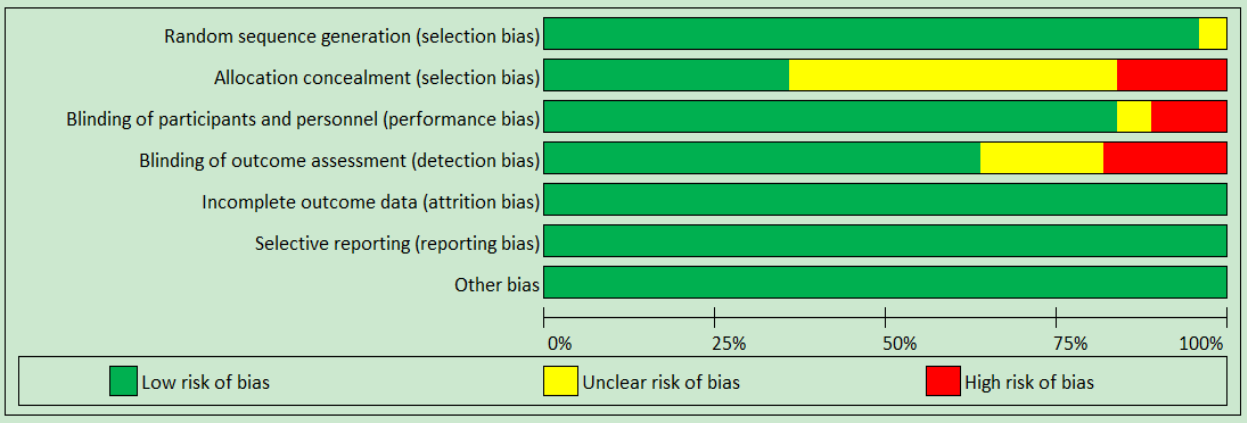


Figure 3: Risk of bias graph - review authors' judgements about each risk of bias item presented as percentages across all included studies.

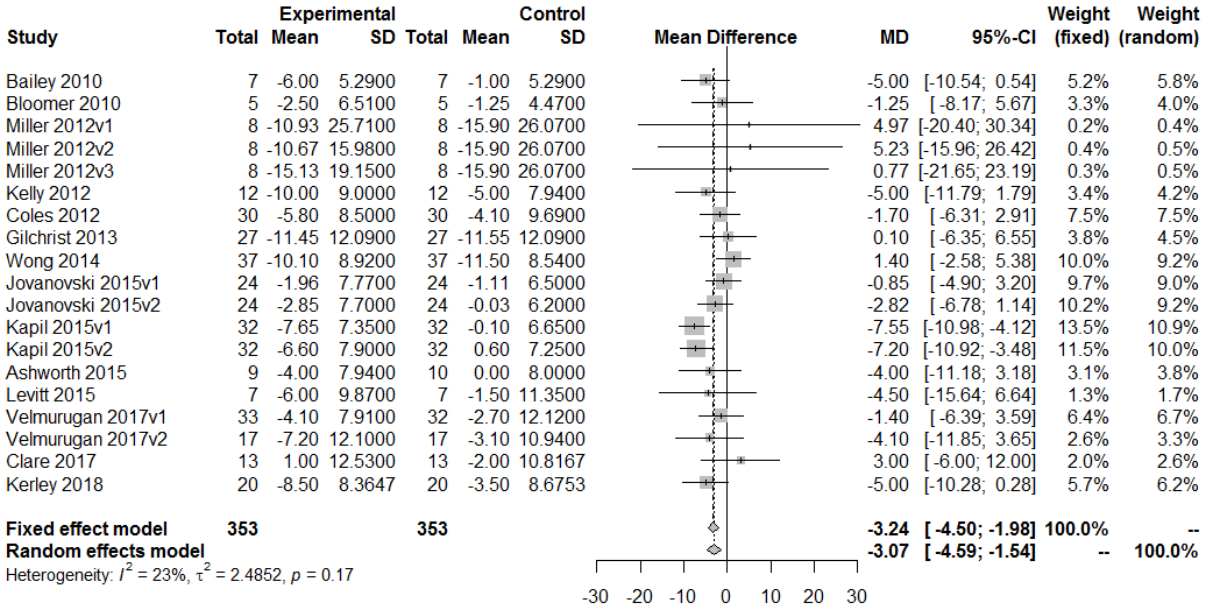


Figure 4: Forest plot of random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on SBP. Both fixed and random effects was estimated to obtain the pooled mean differences in SBP.

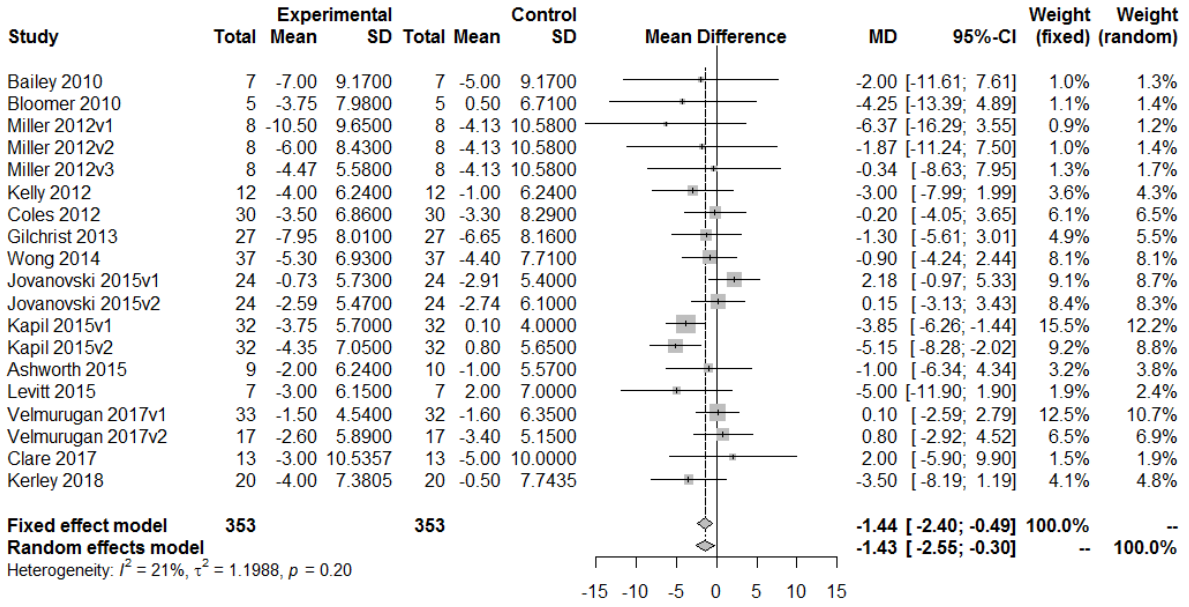


Figure 5: Forest plot of random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on DBP. Both fixed and random effects was estimated to obtain the pooled mean differences in DBP.

Supplementary appendix:

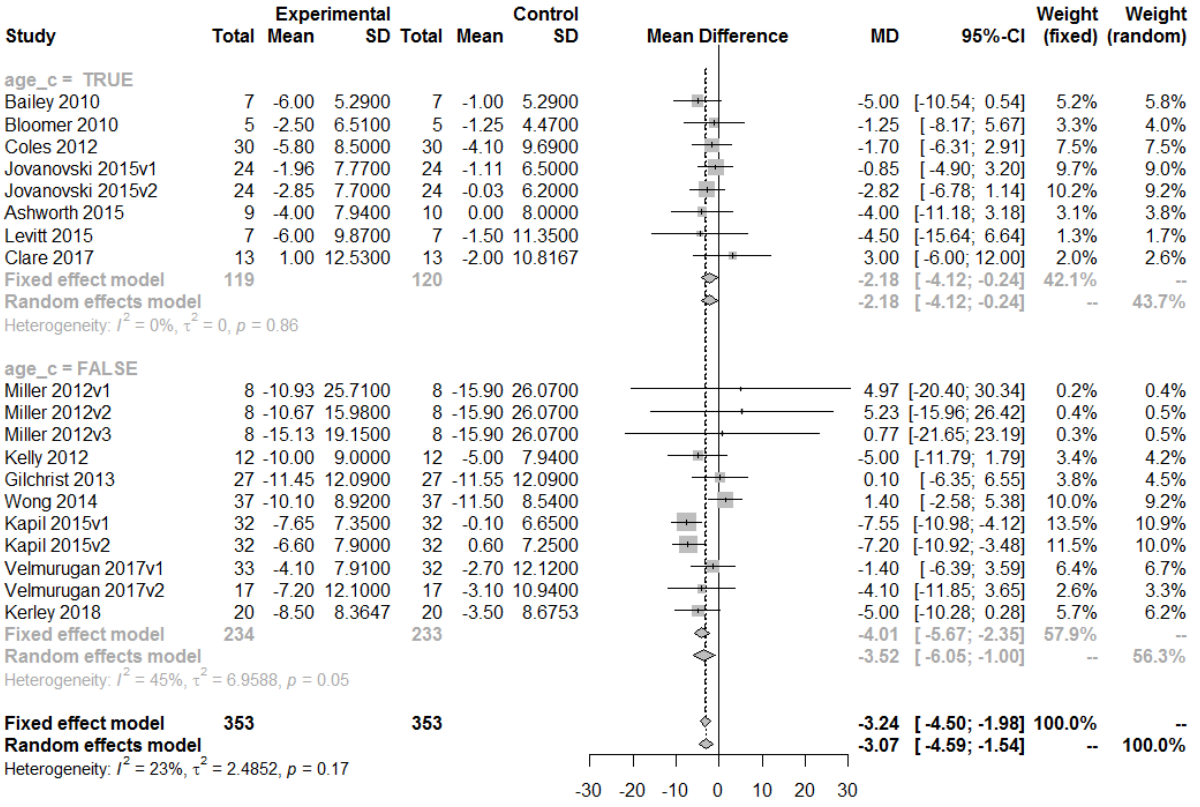


Figure 1 Forest plot of the meta-analysis in random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on SBP was stratified by the age (<50, ≥50 years old). Both fixed and random models were applied to each group to obtain the pooled mean differences in SBP.

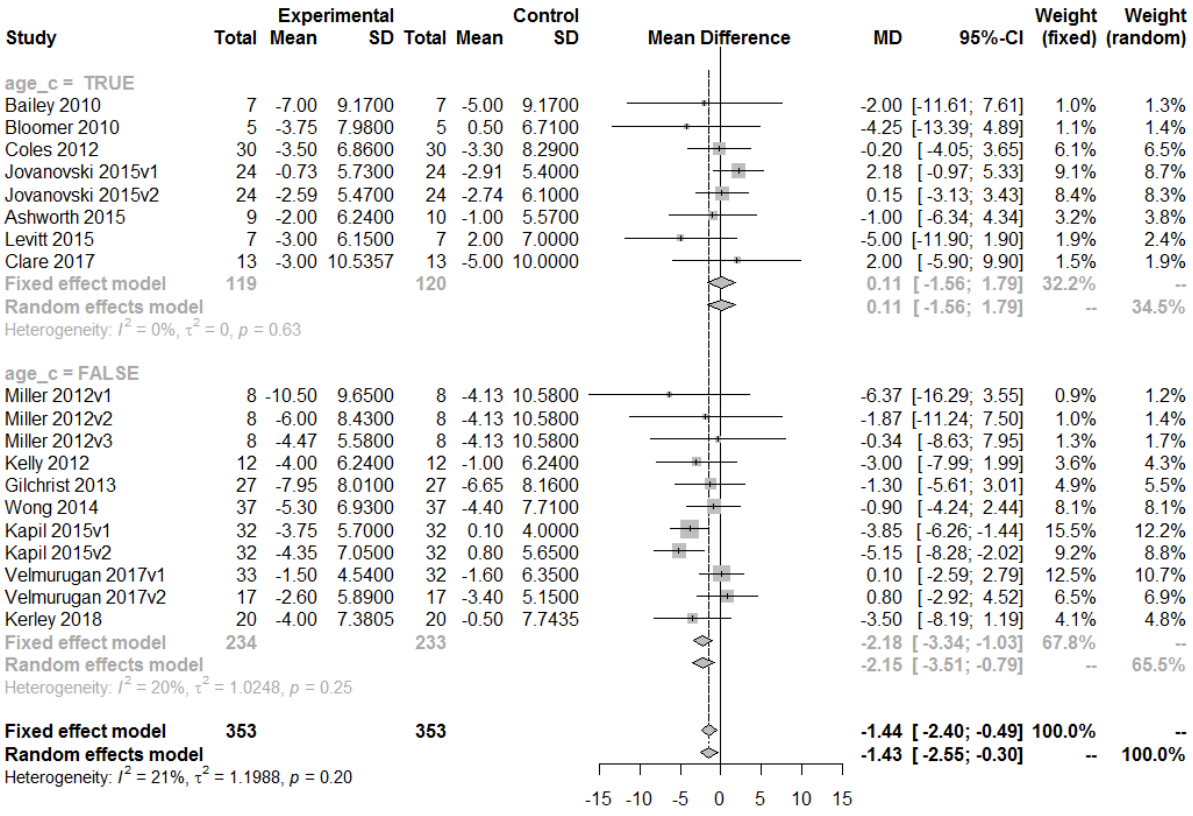


Figure 2 Forest plot of the meta-analysis in random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on DBP was stratified by the age (<50, ≥50 years old). Both fixed and random models were applied to each group to obtain the pooled mean differences in DBP.

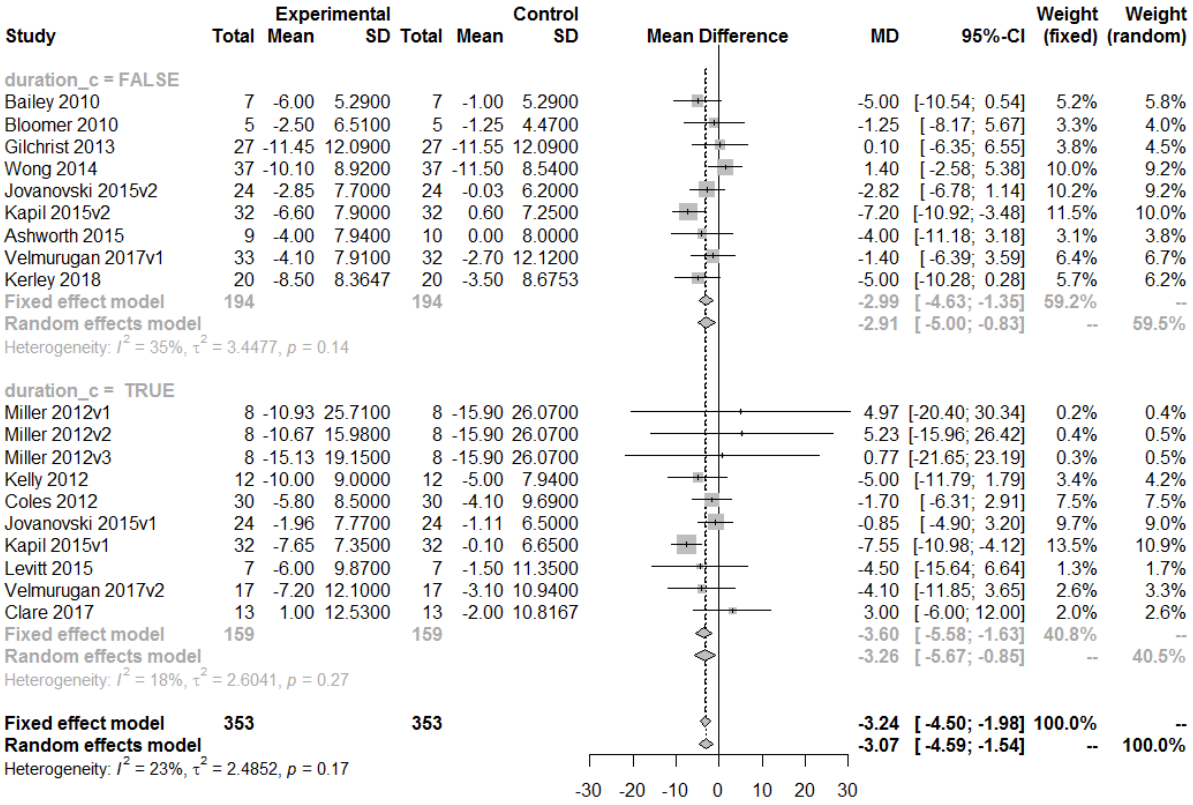


Figure 3 Forest plot of the meta-analysis in random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on SBP was stratified by the study duration (≤72 hours, >72 hours). Both fixed and random models were applied to each group to obtain the pooled mean differences in SBP.

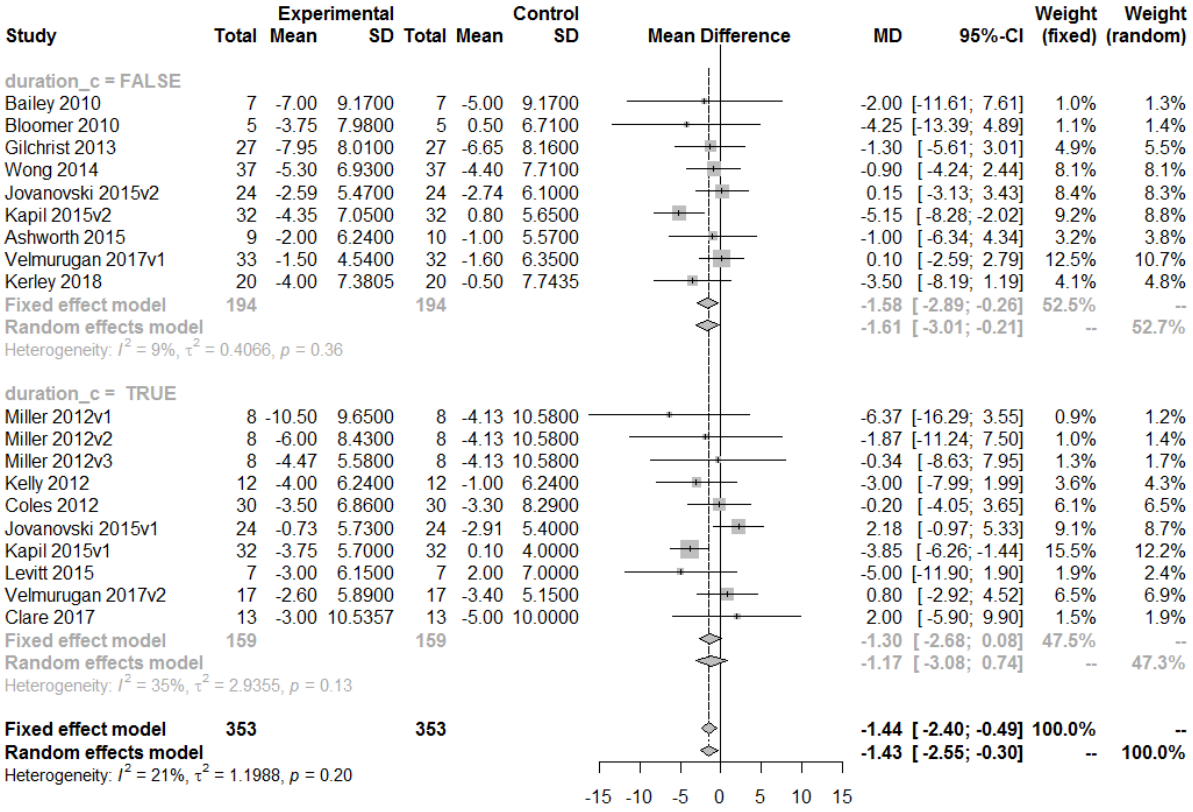
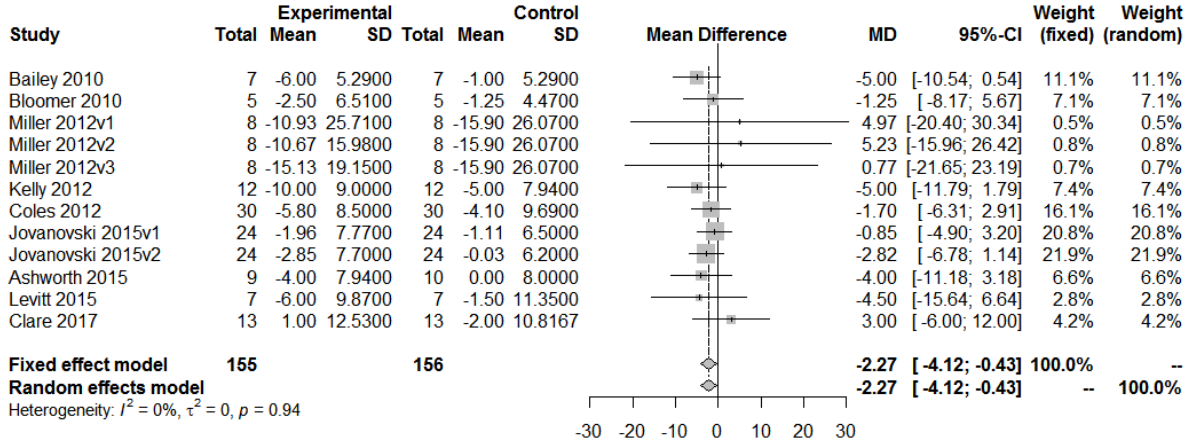


Figure 4 Forest plot of the meta-analysis in random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on DBP was stratified by the study duration (≤72 hours, >72 hours). Both fixed and random models were applied to each group to obtain the pooled mean differences in DBP.



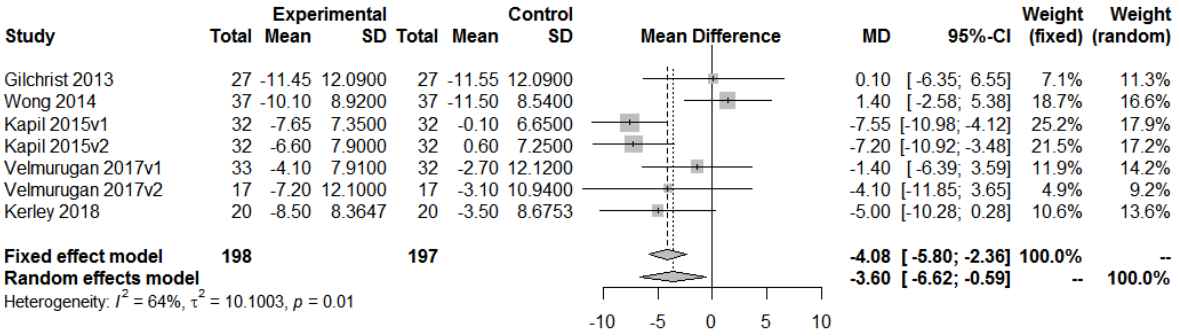
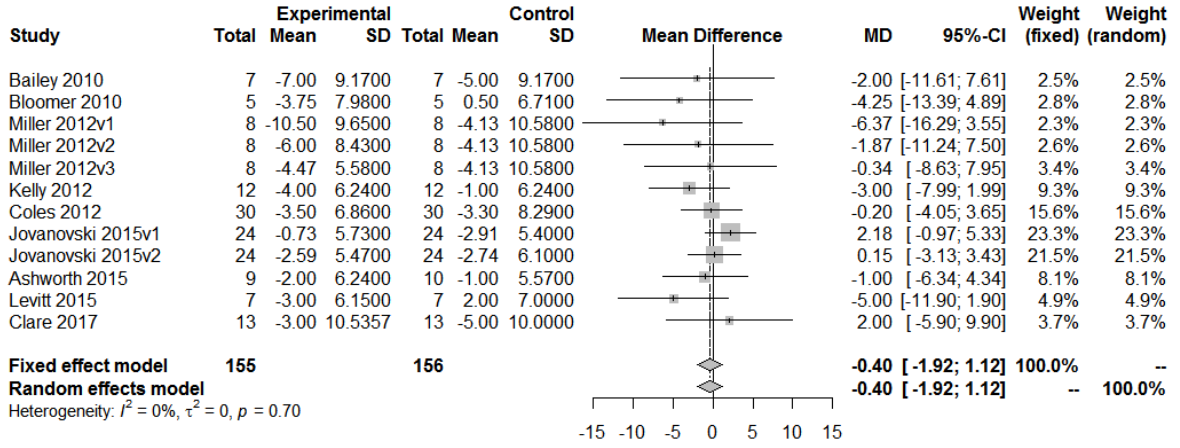


Figure 5 Forest plot of the meta-analysis in random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on SBP was stratified by the healthy conditions (top: healthy, bottom: disease). Both fixed and random models were applied to each group to obtain the pooled mean differences in SBP.



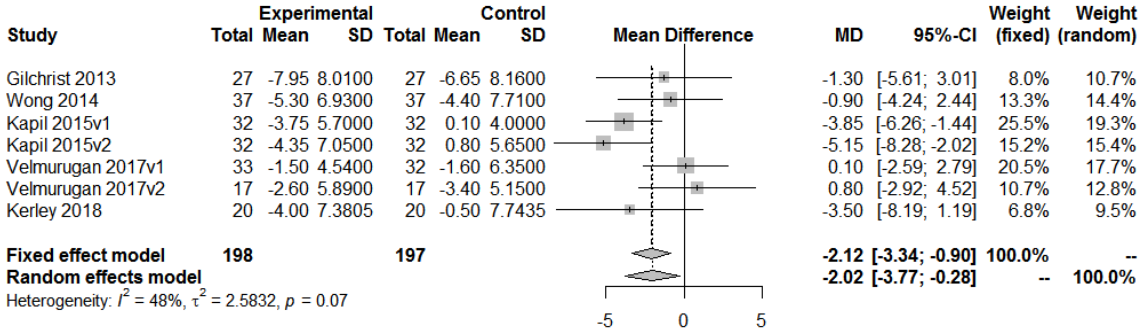


Figure 6 Forest plot of the meta-analysis in random clinical trials investigating the effects of beetroot and inorganic nitrate supplementation on DBP was stratified by the healthy conditions (top: healthy, bottom: disease). Both fixed and random models were applied to each group to obtain the pooled mean differences in DBP.

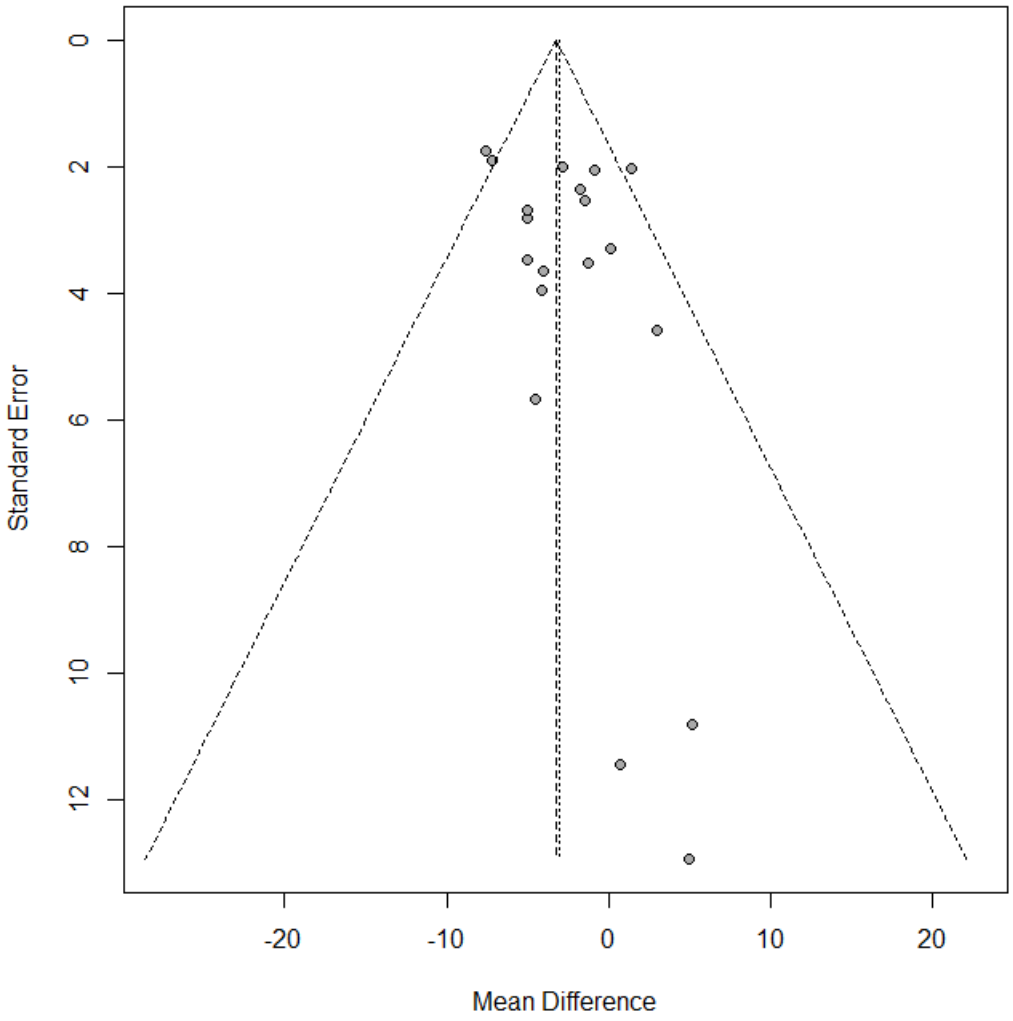


Figure 7 Funnel plot of random clinical trials investigating the heterogeneity of beetroot and inorganic nitrate supplementation on SBP.

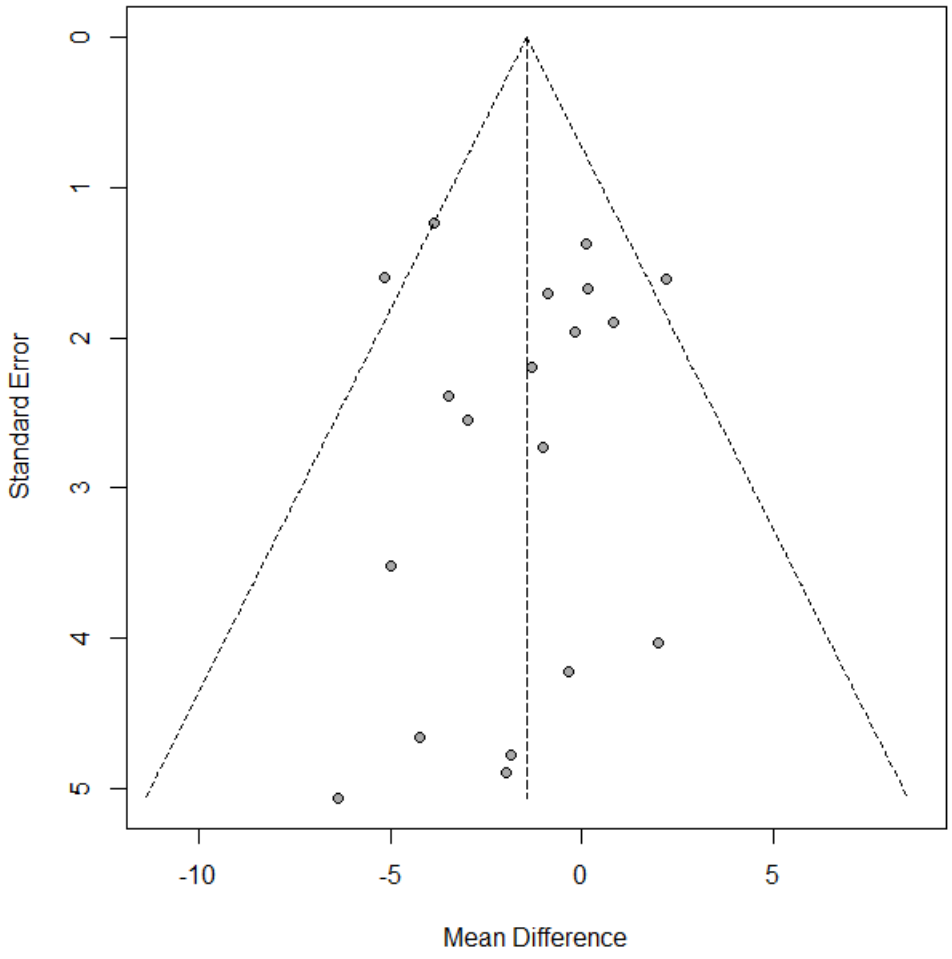


Figure 8 Funnel plot of random clinical trials investigating the heterogeneity of beetroot and inorganic nitrate supplementation on DBP.